Strains of Salmonella enterica Isolated from Abattoirs in Ile-Ife, Osun State, Nigeria

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ABSTRACT

Background: Salmonellosis is one of the most common and widely distributed food borne diseases with tens of millions of human cases occurring worldwide per annum.

Materials and methods: Forty-eight samples were obtained from abattoirs in three local government areas (Ife central, Ife east and Ife north local government areas) out of the four local government areas in Ile-Ife, Osun state, Nigeria. This was between September and November 2018. The samples were transported to the microbiology laboratory, Obafemi Awolowo university, Ile-Ife, Osun state, Nigeria and cultured on pre-enrichment media (Nutrient agar and MacConkey agar) for 18 hours-24 hours at 37°C. They were later transferred into Salmonella-Shigella agar and incubated for 48 hours at 37°C. Isolates were subjected to antimicrobial susceptibility testing on Mueller Hinton medium by disc diffusion method. Results interpretation was done based on the clinical and laboratory standard institute criteria.

Results: Twenty-two Salmonella enterica strains were obtained from a total of 48 samples, representing a prevalence rate of 45.8%. These were: Five strains from Ife central local government with a prevalence rate of 25%; eight strains from Ife east local government with a prevalence rate of 53.3%; nine strains from Ife north local government with a prevalence rate of 69.2%. The strains of Salmonella in this study exhibited multiple-antibiotic resistance. There was however significant susceptibility to Augmentin (Susceptible rate was 54.5%).

Conclusion: Proper hygiene amongst meat handlers should be advocated in the three local government areas. The indiscriminate use of antibiotics should be discouraged in the three localities.

Keywords: Salmonella enterica; Strains; Abattoir

INTRODUCTION

Strains of Salmonella enterica are facultative aerobes. They are gram negative rod-shaped bacteria of the Enterobacteriaceae family. Most serotypes under the species are motile with peritrichous flagellation except Salmonella enterica serovar Pullorum and Salmonella enterica serovars Gallinarum which are non-motile strains as a result of their dysfunctional flagella.

The aim of this study was to determine the hygienic standard of selected abattoirs in Ile-Ife, Osun state, nigeria. The objectives were to: Isolate, identify and characterize strains of Salmonella

enterica from abattoirs in three local government areas (Ife Central, Ife east and Ife north local government areas) in Ile-Ife, Osun state, Nigeria; determine the prevalence rate of the strains in the study area; and determine the susceptibility of the isolated strains to specific antibiotics.

LITERATURE REVIEW

Collection of samples

Forty-eight samples were obtained from abattoirs in three local government areas (Ife central, Ife east and Ife north local

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government areas) out of the four local government areas in Ile-Ife, Osun state, Nigeria. This was between September and November 2018.

Culture

The samples were transported to the microbiology laboratory, department of microbiology, Obafemi Awolowo university, Ile-Ife, Osun state, Nigeria and cultured on pre-enrichment media which were Nutrient agar and MacConkey agar (Oxoid, Ltd, Bashingstoke, Hampshire, England) for 18 hrs-24 hrs at 37°C. They were later transferred into *Salmonella-Shigella* agar (Oxoid, Ltd, Bashingstoke, and Hampshire, England) and incubated for 48 hrs at 37°C.

Identification of isolates

Biochemical tests carried out for the identification of the isolates include: Gram staining; Catalase test; Indole test; Citrate utilization test; Motility test; Methyl Red-Voges Proskauer (MRVP) test; Urease test; Triple sugar iron (Glucose, Lactose, H_2S) test.

Antibiotic susceptibility

Using the method of Adejuwon, et al., all isolates were subcultured on fresh nutrient agar plates using the zigzag method from stock culture and incubated for 24 hrs. Very little amount of the organism was picked and inoculated into appropriately labeled test tubes containing sterile distilled water (5 ml). This was shaken gently to ensure homogeneity. Standardization to 0.5 Mc Farland standard was done. Optical density readings were taken between 0.08–0.1 with the aid of a colorimeter. Using a swab stick, Muller-Hilton agar was inoculated aseptically with each isolate using the spread plate technique. With the aid of a

Table 1: Biochemical tests.

Sample Code **Biochemical Tests**

sterile forceps the multiple antibiotic discs was picked and placed on the surface of each plate. Incubation was for 24 hrs. Zones of inhibition were measured in millimeter with the aid of a ruler before comparing with a CLSI Standard. The antibiotics used were: Septrin (Trimethoprim/Sulfamethoxazole, also known as Cotrimoxazole), Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin, Tarivid (Ofloxacin), Strptomycin [1-5].

Salmonella isolates which showed growth when cultured on nutrient agar, MacConkey agar and Salmonella-Shigella agar (Oxoid, Ltd, Bashingstoke, Hampshire, England) with morphological characteristics specified in the Bergey's Manual of Determinative Bacteriology were obtained. Salmonella enterica isolates were identified. Table 1 shows the biochemical characterization of the isolates. Table 2 shows the morphological reaction of the isolates to the selected media. A total of twentytwo strains of Salmonella enterica were obtained from a total of forty-eight samples collected (representing a prevalence rate of 45.8%). Of these: Five strains of Salmonella from twenty samples were obtained from Ife central local Government (representing 25% prevalence rate in Ife central local government and 10.4% prevalence rate in the total study area); Eight strains from fifteen samples tested were obtained in Ife east local government (representing 53.3% prevalence rate in Ife East local government and 16.7% prevalence rate in the entire study area); Nine strains were obtained from thirteen samples tested in Ife North Local Government Area (representing a prevalence rate of 69.2% in Ife North local government Area and a prevalence rate of 18.7% in the entire study). The prevalence rate of Salmonella enterica in the three local government areas in Ile-Ife, Osun state, Nigeria is represented in Table 3 [6-9].

	Catalase	Oxidase	Indole	Citrate	Motility	Methyl Red	Voges Proskaue	Urease er	Gram Staining	Triple Sugar Iron		on
										Glucose	Lactose	H_2S
NW1	+	-	-	+	+	+	-	-	-	+	-	+
NW2	+	-	-	+	+	+	-	-	-	+	-	+
NW4	+	-	-	+	+	+	-	-	-	+	-	+
NW6	+	-	-	+	+	+	-	-	-	+	-	+
NW8	+	-	-	+	+	+	-	-	-	+	-	+
NW9	+	-	-	+	+	+	-	-	-	+	-	+
NW11	+	-	-	+	+	+	-	-	-	+	-	+
NW14	+	-		+	+	+			-	+	-	+

NW18	+	-	-	+	+	+	-	-	-	+	-	+
NW23	+	-	-	+	+	+	-	-	-	+	-	+
NW24	+	-	-	+	+	+	-	-	-	+	-	+
GLT2(3)a	+	-	-	+	+	+	-	-	-	+	-	+
GLT2(3)b	+	-		+	+	+	-	-	-	+	-	+
GLT10(2)	+	-	-	+	+	+	-	-	-	+	-	+
OSS5(2)a	+	-	-	+	+	+	-	-	-	+	-	+
OSS5(2)b	+	-	-	+	+	+	-	-	-	+	-	+
3	+	-	-	+	+	+	-	-	-	+	-	+
4	+	-	-	+	+	+	-	-	-	+	-	+
5	+	-	-	+	+	+	-	-	-	+	-	+
10	+	-	-	+	+	+	-	-	-	+	-	+
12	+	-	-	+	+	+	-	-	-	+	-	+
19	+			+	+	+	-	-	-	+		+

Table 2: Morphological tests.

Morphological Tests	Results
Growth on nutrient agar	All strains grew on nutrient agar
Gram staining	All strains were gram negative
Growth in Salmonella-Shigella agar	All strains grew on Salmonella-Shigella agar
Motility test	All strains were motile

Table 3: The prevalence rate of Salmonella enterica in three lo	local Nigeria. government areas in Ile-Ife, Osun state
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Location	Sample Code/ Abbatoir	Number of Samples	Number of Isolates	Prevalane in the area (%)	Total prevalance (%)
IFE Central	GLT	10	3	25%	10.40%
	OSS	10	2	-	
IFE East	OIK	5	4	53.30%	16.70%
	ASS	5	2	-	
	AWSS	5	2	-	
IFE North	ODSS	5	4	69.20%	18.70%

KSS	5	3
ESS	3	2

GLT: God's Love Tabernacle Area (Ife Central); OSS: Olowe Slaughter Slab (Ife Central); OIK: Ola Inu Kan Slaughter Slab (Ife East); ASS: Aanuoluwapo Slaughter Slab (Ife East); AWSS: Al-Wajud Slaughter Slab (Ife East); ODSS: Ola Daddy Slaughter Slab (Ife North); KSS: Kobiowu Slaughter Slab (Ife North); ESS: Ebube Slaughter Slab (Ife North)

The results of the susceptibility of the isolates to various antibiotics used are presented in Table 4. Augmentin has proven to be the most effective on the isolates. 54.5% of the total isolates were susceptible to Augmentin. The percentage of the isolates resistant to Tarivid, Pefloxacin, Gentamycin, Chloramphenicol, Amoxicillin, Ciprofloxacin and Augmentin were: 100%, 95.5%, 95.5%, 86.4%, 68.2%, 27.3% and 22.7% respectively [10-12].

Table 4: Antibiotic susceptibility profile of Salmonella enterica isolates.

Antibiotic	Isolates	Susceptible n (%)	Intermediate n (%)	Resistance n (%)	Total n (%)
Septrin	22				
Chloramphenicol	22	0 (0)	3 (13.6)	19 (86.4)	22 (100)
Sparfloxacin	22				
Ciprofloxacin	22	0 (0)	16 (72.7)	6 (27.3)	22 (100)
Amoxicillin	22	0 (0)	7 (31.8)	15 (68.2)	22 (100)
Augmentin	22	12 (54.5)	5 (22.7)	5 (22.7)	22 (100)
Gentamycin	22	0 (0)	1 (4.5)	21 (95.5)	22 (100)
Pefloxacin	22	1 (4.5)	0	21 (95.5)	22 (100)
Tarivid	22	0 (0)	0	22 (100)	22 (100)
Streptomycin	22				22 (100)

DISCUSSION

The isolates of Salmonella in this research investigation were motile gram negative small rods. Grimont and Weill reported similar findings. There seemed to be a high prevalence of Salmonella enterica (45.8%) in the studied environs. Igbinosa and Beshiru reported a higher prevalence (69.44%) in Edo state, Nigeria. In this study, we observed a prevalence of 25% in Ife central local government. From an earlier investigation, Omololu-Aso, et al. reported a lower (40%) prevalence of Salmonella *enterica* in same Ife central local government in 2017. Makut, et al. worked on cow milk products sold in Keffi metropolis, Nasarawa state, Nigeria. They observed a lower prevalence (15.7%) of this isolate in their investigation. According to Bello, et al., contamination of cow meat may arise in the process of removing the gastrointestinal tract during slaughtering.

The Salmonella isolates in this study exhibited multi-antibiotic resistance. The isolates were significantly sensitive to Augmentin. Susceptibility of the isolates to this antibiotic was 54.5%. In 2017, Omololu-Aso, et al. reported a higher susceptibility of Salmonella isolates to Augmentin (80%) from a similar study conducted in Osun state, Nigeria. Tarivid, Pefloxacin, Gentamycin, Chloramphenicol, Amoxicillin and Ciprofloxacin had resistance profiles of 100%, 95.5%, 95.5%, 86.4%, 68.2% and 27.3% respectively for our isolated Salmonella strains. Omololu-Aso, et al. in their previous study observed low resistance of their Salmonella isolates to Tarivid (10%). Makut, et al. worked on cow milk products in Keffi metropolis. They reported that their Salmonella isolates showed 71.4% resistance to Chloramphenicol. This is consistent with the findings of our present study. Igbinosa and Beshiru observed 100% resistance of their Salmonella isolated from abattoirs in Edo state, Nigeria to Gentamycin. This is close to the 95.5% resistance observed in this study.

In Nigeria, many abattoirs dispose their effluents directly into streams and rivers without any form of treatment. The slaughtered animals are washed by the same water. This was observed during the sampling regime of this study. The occurrence of a high total heterotrophic bacteria count and a high Salmonella count in the effluents from such abattoirs poses lots of ecological and epidemiological risks.

Contamination of abattoir surfaces may emanate from the butchers, which could be as a result of using ungloved hands during dressing and processing of the carcass in the slaughtering hall. In addition, the foot wears used by the butchers could function as a vehicle for faecal contamination, since processing and dressing of the carcasses occurs on the open floor in the slaughter houses. Moreover, in developing countries like Nigeria, water bodies such as rivers are used for drinking, bathing, washing, watering of crops and other domestic purposes which also indicate a high impact on the public health of the users. Similar study conducted by Mor-Mur and Yuste indicated that bacteria isolated from wastes and abattoir products include *Pseudomonads*, *Salmonella*, Klebsiella and Proteus.

CONCLUSION

It is concluded from this study that effluents from abattoirs could be potential sources of reservoirs and dissemination of antibiotic-resistant *Salmonella* between animals and humans as well as the environment. The resistance to antibiotics is most likely due to misuse, widespread and lengthy use of antibiotics amongst animals and humans in the study areas.

RECOMMENDATIONS

Regular surveillance network systems to track resistance patterns of *Salmonella* serovars circulating in abattoirs and other

environmental sources should be put in place. Implementing suitable Hazard Analysis and Critical Control Points (HACCP) procedures to decrease the cross-contamination from these environmental sources as well as food animal handlers and the final consumers could decrease the occurrence of multipleantibiotic resistant Salmonella in the environment.

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